

## ELECTRONIC SOMATIC CELL COUNT

Foss 250/300/360/400

(Unless otherwise stated all tolerances  $\pm 5\%$ )

1. Laboratory Requirements (see CP, item 33 & 34) \_\_\_\_\_
  - a. Un-preserved samples may be run up to 72 hours after initial collection \_\_\_\_\_
  - b. Samples may be run up to 7 days after initial collection if preserved with 0.02% 2-bromo-2-nitropropane-1,3-diol (Bronopol<sup>TM</sup>) or 0.05% potassium dichromate ( $K_2Cr_2O_7$ ) \_\_\_\_\_
  - c. Comparative test with DMSCC \_\_\_\_\_
    1. Performed by each analyst performing ESCC test \_\_\_\_\_
    2. Test 4 samples (100K-200K, 300K-500K, 600K-800K and 900K-1.2M) in triplicate for both DMSCC (three separate smears each) and ESCC (three separate sub-samples each, do not read same sample three times) \_\_\_\_\_
    3. Results must be shown to be acceptable prior to official testing by analyst performing comparison, i.e. analyst is not certified until found acceptable. (**co-requisite for certification**) \_\_\_\_\_
    4. Copy of comparison and results in QC record (or easily accessible file in laboratory) \_\_\_\_\_
  - d. Analysts certified in DMSCC \_\_\_\_\_

### APPARATUS

2. See Cultural Procedures, items 1-5 \_\_\_\_\_
3. Automated Electronic Somatic Cell Counters \_\_\_\_\_
  - a. **Fossomatic 250** \_\_\_\_\_
  - a. **Fossomatic 300** \_\_\_\_\_
  - b. **Fossomatic 360** \_\_\_\_\_
  - c. **Fossomatic 400** \_\_\_\_\_
4. Water Bath \_\_\_\_\_
  - a. Circulating and thermostatically controlled to 37-42C \_\_\_\_\_

## REAGENTS

### 5. Stock Dye/Buffer Solution

- a. Dissolve 2.5g (or number of tablets specified by manufacturer) ethidium bromide ( $C_{21}H_{20}BrN_3$ ) in 1 liter deionized (DI) or MS water (caution TOXIC, use gloves when handling and do not breath dust), heat to 40-60C and mix to dissolve
- b. Add 400g tripotassium citrate monohydrate ( $C_6H_5O_7K_3 \cdot H_2O$ ), 14.5g citric acid monohydrate ( $C_6H_8O_7 \cdot H_2O$ ), and 4 liters DI or MS water, heat to 40-60C and mix to dissolve
- c. Add dye and buffer solutions together and mix
- d. Add 50 mL neutral detergent, Triton X-100 to mixture and stir until dissolved
- e. Store refrigerated (0-4.4C) in airtight, light-proof container for no longer than 90 days
- f. Date prep. \_\_\_\_\_ Exp. Date \_\_\_\_\_

### 6. Stock Detergent Solution

- a. Dissolve 10 mL neutral detergent, Triton X-100 in 1 liter of DI or MS water and heat 40-60C to complete solution
- b. Store refrigerated (0-4.4C) in airtight, container for no longer than 30 days
- c. Date prep. \_\_\_\_\_ Exp. Date \_\_\_\_\_

### 7. Ammonium Hydroxide ( $NH_4OH$ ) Solution, Reagent Grade, 25%

### 8. All stock dye/buffer and detergent solutions labeled with date prepared and expiration date

## WORKING SOLUTIONS

### 9. Dye/Buffer Solution

- a. Dilute 1 L dye/buffer stock solution (item 5) with 9 L DI or MS water
- b. Protect from light and use within 21 days
- c. Date prep. \_\_\_\_\_ Exp. Date \_\_\_\_\_

### 10. Rinsing Solution (use within 7 days)

- a. Add 10 mL of stock neutral detergent stock solution (item 6) and 25 mL of ammonium hydroxide solution (item 7) and suspend to 10 L with DI or MS water
- b. Date prep. \_\_\_\_\_ Exp. Date \_\_\_\_\_

11. Optionally, use manufacturer's reagent kits and instructions specific for each instrument \_\_\_\_\_
12. All working dye/buffer and rinsing solutions labeled with date prepared and expiration date \_\_\_\_\_

**START UP**

13. Cell Counter \_\_\_\_\_

- a. Check that the amount of dye/buffer solution (item 9) and rinsing (cleaning) solution (item 10) in instrument supply containers is of sufficient volume for the number of samples to be run \_\_\_\_\_
- b. Solutions not used beyond expiration date(s) \_\_\_\_\_
- c. Turn on power and cycle at least six times \_\_\_\_\_
- d. Perform a zero check before starting any measurements, within acceptable limits, single counts up to 5 and mean up to 3 \_\_\_\_\_
- e. **IF ANY ABOVE PARAMETERS ARE WRONG, CORRECT BEFORE PROCEEDING** \_\_\_\_\_
- f. Records maintained on all parameters each time instrument is used \_\_\_\_\_

14. Milk Standards \_\_\_\_\_

- a. Commercially prepared: \_\_\_\_\_  
Lot# \_\_\_\_\_ Date Rcd. \_\_\_\_\_
1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M \_\_\_\_\_
2. Do DMSCC in triplicate on each standard in set and average counts, records maintained \_\_\_\_\_
3. DMSCC check performed in rotation by all certified analysts \_\_\_\_\_
4. Standards used within one week \_\_\_\_\_
- b. Certified provider: \_\_\_\_\_  
Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_ Date Rcd. \_\_\_\_\_
1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M \_\_\_\_\_
2. Maintain copies of all provided DMSCC values \_\_\_\_\_
3. Measure and maintain records of temperature (0-7.2C) of standards as received \_\_\_\_\_

4. Maintain copies of all correspondence regarding problems \_\_\_\_\_
5. Standards used by manufacturer's expiration date \_\_\_\_\_
- c. Laboratory prepared (weekly) \_\_\_\_\_
  1. Prepare from raw milk > 18 hours old preserved with 0.05% potassium dichromate ( $K_2Cr_2O_7$ ) \_\_\_\_\_
  2. Or, preserved with 0.02% 2-bromo-2-nitropropane-1,3-diol (Bronopol™) \_\_\_\_\_
  3. Standards cannot be preserved with formalin \_\_\_\_\_
  4. Prepare 4 standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M, used within one week  
Date prep. \_\_\_\_\_ Exp. Date \_\_\_\_\_
  5. Do DMSCC in triplicate on each standard and average counts, records maintained \_\_\_\_\_
  6. DMSCC check performed in rotation by all certified analysts \_\_\_\_\_
- d. Hourly Control Sample (instrument drift check) \_\_\_\_\_
  1. Use one of the standards (items 14a or b) in the 500-800K range, run in triplicate and determine average \_\_\_\_\_
  2. Optionally, prepare sufficient control/sample 500-800K range, run in triplicate and determine average \_\_\_\_\_

#### PROCEDURE

15. Testing Standards (each time instrument used) \_\_\_\_\_
  - a. Heat standards to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature, used once and then discarded, i.e. do not re-use \_\_\_\_\_
  - b. Mix by inverting at least 2x, place in rack and put onto automatic track, run within 10 minutes \_\_\_\_\_
  - c. Run the standards six times and average the counts for each level, records maintained \_\_\_\_\_
  - d. Each standard's average must be within 10% of the DMSCC (item 14) for that level, except within 15% for 100K-200K standard, records maintained \_\_\_\_\_
  - e. Repeatability - a standard in the 300K to 800K range must have a coefficient of variation ( $C_v$ ) of 5% or less on 10 replicates (**Refer to Operating Manual**), records maintained \_\_\_\_\_

f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING**

16. Testing Samples

- a. Heat samples to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature; samples must not be re-used and must be discarded after use
- b. Mix by inverting at least 2x, place in rack and put onto automatic track, run within 10 minutes of reaching the testing temperature

17. With continuous operation:

- a. Run a standard or optionally a control/sample (item 14d) in the 500K to 800K range hourly, must be within 5% of the original established instrument average value (optionally, within 10% of original DMSCC average)
- b. Run control 6x
- c. Run zero control (item 13d)
- d. Maintain records

18. Routine maintenance

- a. Perform as described in operating manual
- b. Maintain records

**REPORTS**

19. Computing and Reporting Counts

- a. Count obtained x 1000 is the cell count/mL milk
- b. In reporting electronic somatic cell counts (ESCC/mL), record only first two left hand digits, raising second digit to next higher number when third digit is 6 or more
- c. Report the two left hand digits (rounded)
  - 1. If the third digit is 5 the second digit is rounded by the following rule
    - a. When the second digit is odd round up, raise the second digit by 1 (odd up, 235 to 240)
    - b. When the second digit is even round down, delete the 5 and report the second digit as is (even down, 225 to 220)
- d. If count on instrument is < 100 report as < 100,000 ESCC/mL